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THE FATTY ACIDS IN CORN STARCH AND SYNTHESIS OF CORN BETA AMYLOSE PALMITATE

DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Pure Science of Columbia University.

BY

LEO LEHRMAN, B.S., A.M.

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TO MY
FATHER AND MOTHER

ACKNOWLEDGMENT

The author wishes to express his thanks and gratitude to Professor T. Clinton Taylor, at whose suggestion this investigation was undertaken and under whose guidance it was carried out, for his invaluable advice and hearty co-operation.

THE FATTY ACIDS IN CORN STARCH AND SYNTHESIS OF CORN BETA AMYLOSE PALMITATE

INTRODUCTION AND PURPOSE

Nearly every structural formula so far advanced has assumed starch to be pure carbohydrate without taking into account the occurrence with it of non-carbohydrate substances e. g. phosphoric acid [1, 2, 3] and fatty acids.[4] Taylor and Nelson[4] in this laboratory have shown that after the extraneous fat occurring in corn starch was completely removed by extraction and the whole starch subsequently hydrolyzed by acid, 0.5—0.6% fatty material was liberated. This fatty material they have shown contains palmitic acid and unsaturated substances designated by "X." From their data they have attempted to piece together the linking of this fat with the carbohydrate part of the molecule.

Recently in this laboratory it was shown that corn starch could be separated into two parts—alpha and beta amylose, by either electrophoresis or ultrafiltration.[5] Although both amyloses give glucose, ultimately, on acid hydrolsis the alpha yields also fatty acids. The source of the combined fatty acids, then in whole corn starch is the alpha amylose.

Having for the first time a method available for the production of alpha and fat free beta amylose it was thought desirable to analyze the fatty acid portion of alpha amylose both qualitatively and quantitatively. The next step was to synthesize a simple derivative of beta amylose and one of the identified fatty acids with the idea of simulating, if possible, the properties of the fat bearing alpha amylose. The results of the investigation along these lines are given in this paper.

EXPERIMENTAL

1. The Separation of Unsaturated From Saturated Fatty Acids.

The starting material for this work is "refinery mud"[4] obtained from the Corn Products Refining Company. This "refinery mud" is the solid filtered out of the liquor after the hydrolysis of corn starch in the process of making glucose. It varies in color from grey to dark brown depending on the conditions of filtration at the plant, is wet and has a distinct oily odor.

This material was used as a source for the fatty acids of corn starch because (a) it was a convenient source for large quantities for qualitative analysis, (b) the fatty acids contained in it are the same as those liberated from pure starch as shown later in this article, (c) since the fatty acids in the whole corn starch come from the alpha amylose portion, the fatty acids contained in it are the same as those from pure alpha amylose.

Following the method described by Taylor and Nelson[4] the "mud" was dried and the fat extracted. This fat was a dark brown solid consisting of palmitic acid and an unsaturated part "X." For further reference this will be known as the mixed oil.

In order to have the pure unsaturated material to work with it was necessary to separate the palmitic acid from it. A review of the various methods of separation indicated that the recent work of Thomas and Yu[6] using the magnesium soap-alcohol method was the most adaptable in this case. The magnesium soaps were made and the solid magnesium palmitate filtered off on an alundum cup using suction. The filtrate was distilled in vacuo to get rid of the alcohol, and the liquid residue decomposed by warming with concentrated hydrochloric acid and extracted with petroleum ether. The solution of the unsaturated fatty acids in petroleum ether was allowed to stand over calcium chloride and calcium carbonate (to remove any hydrochloric acid) and then vacuum distilled to drive off the petroleum ether leaving a dark brown oil. Test tube reactions showed that this oil was the same as that de-

scribed by Taylor and Nelson[4] except that it did not show the presence of reducing sugar when treated with Fehling's solution. This whole process was repeated on several batches of "refinery mud" so as to accumulate enough of the unsaturated oil to proceed with the identification.

2. Identification of Unsaturated Fatty Acids.

When unsaturated fatty acids are oxidized under proper conditions two hydroxyl groups are introduced at each double bond forming a saturated compound which generally is solid. This was the first method of attack and accordingly some of the unsaturated oil was oxidized by alkaline potassium permanganate solution.[7] The precipitate was filtered off and set aside.

These acids were separated by taking advantage of the solubility of the one and the relative insolubility of the other acid in ether. After several washings of the mixture with ether and several recrystallizations of the ether soluble portion from ether and alcohol, a white solid (needles under the microscope) was obtained, m.p. 128—129° C.[8, 9, 10, 11] Following is the results of the combustion analysis:

Weight of sample	Weight of CO_2	Weight of water	% Carbon	% Hydrogen
0.1234 gms.	0.3094 gms.	0.1249 gms.	68.39	11.33
0.1126 "	0.2812 "	0.1152 "	68.11	11.37
Theoretical for $C_{18}H_{34}O_2(OH)_2$ (dihydroxystearic acid)			68.34	11.48

From the above data it is seen that the compound is dihydroxystearic acid derived from oleic acid in the unsaturated oil.

The ether insoluble part was boiled up with water, filtered hot and allowed to cool slowly whereupon a silky white solid deposited. This was filtered off, recrystallized from water and dried. This solid had a needle formation under the microscope, m.p. 155—156° C.[12, 13, 14] Following is the results of the combustion analysis:

Weight of sample	Weight of CO_2	Weight of water	% Carbon	% Hydrogen
0.1056 gms.	0.2401 gms.	0.0971 gms.	62.00	10.22
0.1148 "	0.2620 "	0.1066 "	62.24	10.32
Theoretical for $C_{18}H_{32}O_2(OH)_4$ (sativic acid)			62.02	10.42

From the above data it is seen that the compound is tetrahydroxystearic (sativic) acid derived from linolic acid in the unsaturated oil.

The filtrate from the mixture of the hydroxy acids was examined now for the presence of higher hydroxylated acids which would indicate higher unsaturated fatty acids in the original unsaturated oil but there were no indications of such substances.

A modified oxidation method according to Fahrion[12] was tried on another sample of the unsaturated oil yielding the same results as above.

It is a well known fact that unsaturated acids will add bromine at the double bond forming saturated bromo compounds many of which are crystalline and have definite melting points. This offered a means of checking further the acids found by the oxidation method. Some of the mixed oil then was brominated [15] but the yield of bromo compounds was so small it was decided to use the unsaturated oil. After bromination no precipitate of ether insoluble bromo compounds was present indicating the absence of acids more unsaturated than linolic and verifying the result as obtained by the oxidation method. Further treatment of the solution yielded a white solid which, after several recrystallizations from petroleum ether, showed a needle structure under the microscope and m.p. 113° C.[16] Determinations of bromine by the Carius method yielded the following results:

Weight of sample	Weight of Ag Br	% Br
0.1658 gms.	0.2071 gms.	53.15
0.1944 "	0.2439 "	53.38
Theoretical for $C_{18}H_{32}O_2Br_4$ (tetra brom stearic acid)		53.33

From the above data it is seen that the compound is linolic tetrabromide which must be derived from linolic acid in the unsaturated oil.

The filtrate from the separation of the tetrabromide was examined now for other bromo compounds because of doubtful purity of the liquid dibromide from oleic acid. It would not be valid to take a quantitative estimation of bromine on

this liquid residue as a criterion for the presence of the oleic dibromide as the occurrence of any unbrominated oil, or saturated fatty acid would materially affect the result. However, should more than a trace of bromine be indicated as present in compound formation this would be good evidence that the dibrom compound was there. Accordingly a portion of the liquid was boiled with fuming nitric acid in the presence of solid silver nitrate and on dilution with water a fair amount of silver bromide precipitated showing that the oleic dibromide was undoubtedly formed.

When oleic acid is treated with nitrogen trioxide it is gradually changed into the isomeric body elaidic acid, which is solid at room temperature. Higher unsaturated fatty acids are not affected in the same way.[16, 17] It was decided to try this reaction on the unsaturated oil so as to get additional evidence as to the occurrence of oleic acid. Allen[17] recommends the use of a freshly prepared solution of mercurous nitrate due to its power to retain nitrous acid and his procedure was followed, the mixture being stirred every ten minutes. The following shows the course of the reaction:

Time	Color of Precipitate	Amount of Precipitate
Addition of Reagent	brown	
30 minutes	reddish brown	⅓ of test tube
45 minutes	light brown	whole test tube but not solid
90 minutes	yellow	material was completely solid

The results of the above reaction show the presence of oleic acid in the unsaturated oil. Linolic acid under these conditions remains unchanged.[16, 17]

At this point, from the data obtained, the presence of oleic and linolic acids have been shown in the unsaturated oil and the absence of any other fatty acids.

4. Examination for other substances.

The next step was to find out if any substances other than fatty acids were present in the unsaturated oil. Accordingly a sodium fusion[18] was made on the mixed oil so as to be sure

to include anything that might remain with the saturated fatty acid in the separation. This test gave negative results for nitrogen, sulphur and halogens.

A fusion with a mixture of equal parts of sodium carbonate and sodium nitrate[19] and the mixed oil in a platinum crucible showed the absence of phosphate ion and therefore the absence of any organic phosphorus compounds in the original mixed oil.

The only other likely possibility judging from the history and nature of the material was phytosterol. The usual method of determining this substance is the solution of the unsaponifiable matter in ether and the examination of the ether soluble residue.

The improved method of Kerr and Sorber[20] was used on the mixed oil and the results were negative. During the alcoholic saponification of the mixed oil necessary in the separation of saturated from unsaturated fatty acids no unsaponifiable matter was ever seen but often small amounts of the sterols will remain in solution or form an emulsion with the soap[21] solution. Therefore in order to detect traces the Liebermann-Burchard[22] reaction was applied but this gave negative results.

Summing up, from the evidence, it can be said that the fatty material obtained by extracting the solid material resulting from the hydrolysis of corn starch consists of a mixture of palmitic, oleic and linolic acids.

5. Quantitative Determination of Fatty Acids.

After the hydrolysis of the corn starch suspensions in the autoclave, plant practice is to filter the liquid through a steam heated filter-press. Often the "mud" becomes contaminated with iron soap from the autoclave and slightly oxidized due to the hot press. To avoid this a batch of "mud" was obtained just prior to being put through the filter press. It had a greyish color and contained more water than the usual sample.

The "mud" was dried and the fatty acids extracted and separated in the same way as described in Part 1. Iodine

numbers were made then on the mixed and unsaturated oils according to the Hanus method.[23] The solutions were standardized against resublimed iodine and the time of contact between oil and iodine solutions was changed to 60 minutes because experiment had showed this modification was essential in order to obtain concordant results and to check standard oleic acid. The accuracy of the modified method was now determined on a sample of pure oleic acid $d^{22^0} = 0.893$, refractive index $^{22^0} = 1.461$ made from a commercial sample of "pure" oleic acid by forming the magnesium soap in alcohol[6] and decomposing with hydrochloric acid. Following is a table of the results of iodine number determinations:

Weight of oleic acid	Time	Iodine number
0.1765 gms.	60 minutes	90.1
0.1842 "	60 "	90.3

The theoretical iodine number of oleic acid is 90.07[7] so that our results as an absolute value apparently are valid. The next step was to determine the correct values for both the mixed and unsaturated oils using the modified method. A fresh sample of "mud" was dried, extracted and the fatty acids separated as previously described. Following is a table of the results:

Kind of oil	Weight of oil	Time	Iodine number
Mixed	0.1898 gms.	60 minutes	98.54
Mixed	0.2079 "	60 "	98.78
Unsaturated	0.1966 "	60 "	132.90
Unsaturated	0.1386 "	60 "	133.56

At this time Rosenmund and Kuhnhenn[24] brought out a new method for determining iodine numbers in fats and oils by means of pyridine sulphate dibromide. They showed that by use of their reagent the bromine was added on but no substitution or oxidation took place. In their paper they give a comparison of values on known fats between their method and the Hanus method for different times of contact of the two solutions. A survey of their results shows that the value ob-

tained by both methods are nearly the same, especially those in which reagent and fat were in contact for fifty minutes. This is further evidence for the correctness of the iodine number as obtained by this method.

In order to be sure that all of the fatty acids had been liberated from the corn starch and no unhydrolyzed compounds remained, separate portions of the "mud" were boiled up successively with 10% solutions of hydrochloric acid and potassium hydroxide for 30 and 45 minute intervals and the iodine numbers of the mixed and unsaturated oils extracted from the treated "mud" were determined. There was no marked change in the iodine values. As such a large amount of "mud" is used in each recovery of fat, the mixed and unsaturated oil portions were given the same acid and alkali treatment but again the iodine numbers remained about the same.

The mixed oil was methylated [25] and then distilled in vacuo at 1-2 m.m. pressure catching the portion coming over from 155—175° C. This was done to separate, if possible, the fatty acids' esters from one another and from other compounds which might have been present. Following is a table of the iodine values of the separated methyl esters:

Weight of methyl ester	Iodine number
0.1540 gms.	116.90
0.1569 "	116.54

Although the distillation of the methyl esters did not give a clean cut separation, a fact previously noticed by other investigators,[25, 26] there was no indication of substances other than the methyl esters of the three fatty acids mentioned above.

In order to be certain that the results are on pure fat from the corn starch molecule, it was decided to obtain the fatty acids directly from the corn starch. A special alkali washed starch showing .02% extraneous extractable material with petroleum ether was used. Ten pounds of this was hydrolyzed [4] and the liberated fat extracted. The mixed oil was a light yellow colored solid having an iodine value of 101.5 and is free from nitrogen, phosphorus and unsaponifiable mat-

ter. The saturated and unsaturated acids were separated this time by Holde's[27] thallium soap-alcohol method. The unsaturated portion was a light yellow liquid with an iodine value of 133.5. Following is a table of results:

Kind of oil	Weight of oil	Iodine number
Mixed	0.1753 gms.	101.5
"	0.2620 "	101.4
Unsaturated	0.1490 "	133.3
"	0.1336 "	133.6

Knowing that the unsaturated oil apparently consists of only two fatty acids the amount of each can be determined by solving the following equations:

$$x + y = 100$$

$$\frac{90.07x}{100} + \frac{181.42y}{100} = I$$

where x and y are the amounts of oleic and linolic acids respectively; 90.07 and 181.42 are the theoretical iodine numbers of these two fatty acids and I is the iodine value (133.5) of the mixture. Substituting the correct value for I and solving the quadratic equations

$$x = 52.46\% = \text{oleic acid}; \quad y = 47.54\% = \text{linolic acid}$$

in the unsaturated oil. Similarly the following equations represent the mixed oil:

$$w + z = 100$$

$$\frac{Ow}{100} + \frac{133.5z}{100} = I$$

Where w=amount of palmitic acid in mixed oil
z=amount of mixture of oleic and linolic acids in the mixed oil
I=iodine value of the mixed oil (101.5)

133.5 being the iodine value of the mixture of oleic and linolic acids, palmitic acid having an iodine value of zero. Substituting the correct value for I and solving the equations

w=23.97%=palmitic acid;
z=76.03%=oleic+linolic acids.

Knowing the ratio of oleic to linolic acid the final composition of the fatty part of the corn starch molecule from the data obtained is as follows:

Palmitic acid = 24%
Oleic " = 40%
Linolic " = 36%

6. Beta-Amylose Esters of Palmitic Acid.

HISTORICAL.

Berthelot[28] in 1856 was the first one to attempt seriously to make any fat carbohydrate compounds and study them. Briefly stated he heated carbohydrates and fatty acids to high temperatures for several hours but the yields were small and the compounds impure.

Bloor[29] was looking for a fat which would give some information with regard to fat transportation in the blood stream. He heated mannitol with stearic and lauric acids separately in the presence of concentrated sulphuric acid isolating mannid distearate, mannitan distearate, isomeric mannid distearate and the corresponding laurates.

Levene in a series of researches investigated substances occurring in the animal world for fatty acids in combination. He was able to identify both saturated and unsaturated fatty acids in cerebrosides,[30] liver lecithin, [31] egg yolk lecithin,[32] brain cephalin[33] and lecithin.[34]

Zemplen[35] in 1915 prepared a number of penta-acyl derivatives of dextrose by the action of acid chlorides of higher fatty acids in the presence of dry pyridine in very cold chloroform.

Hess and Messmer[36] in 1921 made a number of acyl derivatives of various sugars by using the chloride of both the

lower and higher homologues of the fatty acids at —15° C. They suggested a structure for cellulose similar to the fats and mentioned the fact that it is possible that there are naturally occurring fats in which the sugars play the role of glycerole.

Lapworth and Pearson[37] made oleate and stearate derivatives of mannitol by heating the natural fats with mannitol in the presence of sodium ethoxide as a catalyst. These compounds were similar to those previously made by Bloor.[29]

Irvine[38] and Gilchrist in 1924 state that with the exception of starch containing combined palmitic acid (Taylor and Nelson[4]) no natural compounds are described in which acyl residues characteristic of fats substitute hydroxyl groups of carbohydrates. They made a synthetic fat containing one oleyl group by the action of heat on a mixture of alpha-methyl glucoside and olive oil in the presence of sodium alkoxide as a catalyst. By a series of reactions in which they identified one of the later products, they were able to reason back and thus determine the position of the oleyl group in the compound. Starting with the fat prepared by Lapworth and Pearson[37] they carried out similar reactions and determined the configuration of the dioleate of mannitol.[39]

Recently several investigators[40, 41, 42] have made fat carbohydrate compounds by treating the carbohydrate (starch, cellulose and sugars) with the fatty acid chloride in the presence of a suitable solvent as chloroform, toluene, etc., and a base such as pyridine or quinoline which acts as a promoter of the reaction.

a. Attempt to Synthesize Beta-Amylose*-Fat Compound.

Irvine[38] in his work on the preparation of a synthetic fat containing a methyl glucoside residue introduces a fatty acid molecule into the carbohydrate molecule by heating with a triglyceride in the presence of sodium alkoxide as a catalyst.

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* The authors wish to thank Dr. H. A. Iddles of Columbia University, for his kindness in supplying all the Beta-Amylose used in this work.

All these investigators in making esters, have used the whole starch which is a heterogeneous mixture, while our purpose is to introduce the palmitic acid only into the pure carbohydrate portion of the corn starch, namely, the beta-amylose. However, instead of using the triglyceride which meant that a large amount of glycerine would later be liberated, methyl palmitate was employed. The methyl palmitate was made from the palmitic acid obtained from the mixed oil of the "refinery mud" by making an alcoholic solution, chilling and filtering off the palmitic acid.

The actual procedure, a modification of that used by Irvine,[38] was as follows: 5 grams of methyl palmitate and 1.5 grams of B-Amylose were heated for three hours at 150° ± 10° C. The sodium alkoxide was purposely omitted so as to prevent the possibility of soap formation. The liquid on top was poured off and the solid on the bottom refluxed with ether and petroleum ether so as to remove any extraneous fat. The residue was then hydrolyzed with hydrochloric acid and any fat liberated extracted with petroleum ether. No extractable matter was obtained even when the time of heating was raised to 8 hours, showing that no compound had been formed. If the temperature is raised the product darkens (dextrinizes) but even this did not help in forcing compound formation.

The process was repeated with the addition of 0.25 grams of $NaOCH_3$. Before hydrolyzing, the residue was refluxed with 95% alcohol til a small sample on ignition left no ash showing that any soap formed in the process was removed. The solid material free from soap and extraneous fat was hydrolyzed and the resultant solution extracted with petroleum ether but again no fat was found. This method of making a fat-carbohydrate compound was abandoned.

b. Modified Method of Making Palmityl Chloride.

Recently Gault[40] and Karrer[41] prepared fat-carbohydrate compounds such as starch hexa-palmitate, starch hexa-laurate, cellulose hexa-stearate by treating the carbohydrate with the higher fatty acid chlorides in the presence of a solvent such as chloroform or toluene and promoted by the addition of a base

as pyridine or quinoline. It was decided to try this method using palmityl chloride and B-Amylose. The only reference to the preparation of palmityl chloride was that of Krafft and Burger,[43] who made it by treating palmitic acid with phosphorus pentachloride and distilling the reaction product in vacuo. Several attempts to duplicate their work were unsuccessful. It seemed that the phosphorus penta-chloride was too active a chlorinating agent, side reactions taking place with the result that on distilling, even under reduced pressure, a tar was obtained in the distilling flask. Knowing that phosphorus trichloride is used in preparing the chlorides of the lower fatty acids where a less energetic chlorinating agent is required, it was tried on palmitic acid. The method was as follows: To 25 grams of pure palmitic acid, 10 grams of phosphorus trichloride was slowly added mixing the two together intimately (Note: It is essential that the phosphorus trichloride should be freshly redistilled as several attempts with a material that had been standing for some time gave negative results). The mixture which warms up a little is put on a steam bath for three hours to help the reaction along. At first a homogenous light yellow liquid is formed but after a short time a second heavier liquid layer appears. As the heating goes on this second layer increases in volume and an orange colored precipitate begins to settle on the bottom. At the end of the three hours heating the upper liquid layer is poured off into a distilling flask and distilled in vacuo (15 m.m.) catching a watery white distillate between 190-205° C. and leaving a tarry residue in the distilling flask. This distillate exhibited the reactions of an acid chloride and on analysis by saponification with potassium hydroxide showed 12.77% Cl, the theoretical value for palmityl chloride being 12.90% Cl. Yield 10 grams. It was found however, that the liquid from the reaction without distilling could be used if the slight amount of phosphorus trichloride, which distilled at a much lower temperature in vacuo than the palmityl chloride, was removed thus increasing the yield.

c. Preparation of a Fat-Carbohydrate Compound.

Following Gault's[40] method with slight modifications 2 grams of dry B-Amylose, 6 c.c. dry pure pyridine, 10 c.c. dry pure toluene and 10 grams of palmityl chloride were mixed together and heated on a steam bath for 5 hours. During the course of the reaction the B-Amylose softens and some of it goes into solution. At the end of the heating a small amount of a sticky solid (which will be spoken about later) was left on the bottom. The upper yellow liquid was poured into alcohol getting a white precipitate which was filtered, dissolved in chloroform, re-precipitated with alcohol, filtered and dried. This substance is a horny like material having no crystalline form soluble in chloroform, benzene and ether; insoluble in alcohol and water; softens at 50° C. and melts at 75° C. It gives no color with iodine. The amount of fat in the compound was determined by saponifying with aqueous alkali and found to be 84%.

It is seen that the substance has such a high percentage of fat that it has taken on the properties of a fatty substance and its carbohydrate character has been masked. Our purpose was to make a compound that would have a fat content near 3% [5] so that it would be similar in fat content to alpha amylose.

d. Preparation of a Fat-Beta Amylose Compound having a Fat content similar to that of Alpha Amylose.

The small amount of sticky solid left over from the previous experiment was analyzed for fat content and gave a result of 12.5%. This gave a clue that by varying the conditions and concentrations it might be possible to obtain a compound having a low fat content. After a number of attempts the following method was worked out: 5 parts of dry ground B-Amylose, 6 parts dry pyridine, 10 parts dry toluene, 5 parts palmityl chloride were mixed and heated on a water bath for about 15 minutes. The liquid was poured off and the residue boiled with two portions of chloroform decanting each time. The residue was next boiled with alcohol also decanting and

finally dried in an oven. It is very difficult to get the material free from pyridine. The dried substance was suspended then in water and electrophoresed in an apparatus similar to that used by Iddles.[5] A short time after the current was turned on the suspension began to form flocks and gradually moved downward toward the anode. Previous tests in a U tube had shown that this material acted like alpha amylose in migrating to the anode. When the supernatant liquid was clear the current was turned off and this clear solution tested with iodine. If a color reaction took place the solution was siphoned off and fresh distilled water put in and the solid material stirred up in it. During the electrophoresis pyridine began to accumulate in the cathode compartment so the liquid was changed frequently and distilled water added. This procedure was repeated till the top clear solution gave no color with iodine. In the meantime the pyridine had also been removed. The solid on the bottom was removed, frozen,[5] filtered after warming up and the residue washed with absolute alcohol and ether.

In order to be certain that the polar character of the beta amylose palmitate was due to the introduction of the fatty acid radical and not to any changes brought about by the pyridine, some beta amylose was carried through the above operation but the palmityl chloride was omitted. A gelatinized paste of this material in water did not migrate when placed in the cell showing that it was non-polar just as beta amylose is non-polar.

The substance which migrated is a white, starchy looking substance, insoluble in all organic solvents, forms a turbid solution in water which gives a light blue color with iodine. A sodium fusion showed this compound to be free of nitrogen. Following is the result of fat by hydrolysis[4] on this compound from different electrophoresing experiments:

	Weight of substance	Weight of fat	% fat
1st run	2.8654 grams	0.1994 grams	6.96
2nd run	2.6732 "	0.1802 "	6.74
3rd run	3.4950 "	0.2447 "	7.00

This compound resembles the alpha amylose in two respects:

1. It has fat combined with it.
2. It migrates to the anode when aqueous suspensions are electrophorised.

SUMMARY.

1. The unsaturated part of the fat in the corn starch molecule is a mixture of oleic and linolic acids.

2. The absence of other fatty acids and substances like sterols, etc., has been established.

3. The fatty part of the corn starch molecule is composed of

Palmitic acid	= 24%
Oleic "	= 40%
Linolic "	= 36%

4. A new ester of beta-amylose and palmitic acid was made uncontaminated with derivatives of the alpha portion.

5. A study of its properties shows it to resemble to some extent alpha amylose* and indicates that the introduction of fatty acids into a non-polar amylose causes it to become polar.

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* Results of investigation now in progress in this laboratory on the structure of corn alpha amylose indicates that the fatty acids are held in a manner different from that in the above synthetic compounds so the latter compounds should be expected to resemble only slightly the natural corn alpha amylose.

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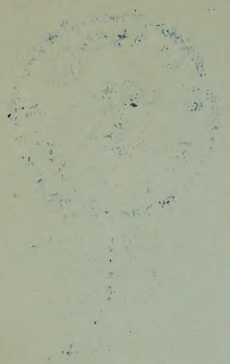

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